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| EXAMINER |
|---|
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| ART UNIT | PAPER NUMBER |
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Please find below and/or attached an Office communication concerning this application or proceeding.

| Office Action Summary | Application No. | Applicant(s) | |
|---|---|---|---|
| | 10/056,794 | GRAVES ET AL. | |
| | Examiner | Art Unit | |
| | David J Blanchard | 1642 | |

*-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --*

**Period for Reply**

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

**Status**

1)☐ Responsive to communication(s) filed on _____ .

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

**Disposition of Claims**

4)☒ Claim(s) *11-14,16,51 and 52* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *11-14,16,51 and 52* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

**Application Papers**

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

**Priority under 35 U.S.C. §§ 119 and 120**

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☒ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

**Attachment(s)**

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449) Paper No(s) *1.5 & 8* .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☒ Other: *See Continuation Sheet* .

Continuation of Attachment(s) 6). Other: Notice to comply with sequence requirements.

## DETAILED ACTION

### *Specification*

1. The amendment filed in paper number 7 on 9/24/02 is objected to under 35 U.S.C. 132 because it introduces new matter into the disclosure. 35 U.S.C. 132 states that no amendment shall introduce new matter into the disclosure of the invention. The added material, which is not supported by the original disclosure is as follows: The amendment claims priority to Application No. 08/871,488, filed on June 9, 1997, which issued as U.S. Patent Application No. 6,358,710; which application is a continuation-in-part of U.S. Patent Application 08/660,362, filed on June 7, 1996 and abandoned. The priority applications were not incorporated by reference in the originally filed instant application. The incorporated by reference of the priority applications in the amendment filed in paper number 7 on 9/24/02 constitutes new matter. This objection can be overcome by removing the incorporation by reference statement.

   Applicant is required to cancel the new matter in the reply to this Office Action.

2. Claims 11-14, 16, 51 and 52 are pending and under examination.

### ***Sequence Requirements***

3. In order to have compact prosecution a first office action can be performed on this application, however, this application contains sequence disclosures that are encompassed by the definitions for nucleotide and/or amino acid sequences set forth in 37 C.F.R. § 1.821(a)(1) and (a)(2). This application fails to comply with the requirements of 37 C.F.R. §§ 1.821-1.825. Although the claims in the instant application are not drawn to specific sequences, the disclosure contains sequences that need SEQ ID numbers on page 52, lines 26, 27 and page 53, lines 8, 9, 18, 19 and page 54, lines 22, 25 and page 55, lines 8, 10 and page 63, lines 3, 4, 13, and 14. Applicant is reminded to check the entire disclosure to ensure that the application is in sequence compliance.

4. Any questions regarding compliance with the sequence rules requirements specifically should be directed to the departments listed at the bottom of the Notice to Comply.

5. APPLICANT IS GIVEN THE TIME ALLOTED IN THIS LETTER WITHIN WHICH TO COMPLY WITH THE SEQUENCE RULES, 37 C.R.F. §§ 1.821-1.825. Failure to comply with these requirements will result in ABANDONMENT of the application under 37 C.F.R. § 1.821(g). Extensions of time may be obtained by filing a petition accompanied by the extension fee under the provisions of 37 C.F.R. § 1.136. In no case may an applicant extend the period for response beyond the six-month statutory period. Direct the response to the undersigned. Applicant is requested to return a copy of the attached Notice to Comply with the response.

***Claim Rejections - 35 USC § 112***

6. The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter, which the applicant regards as his invention.

7. Claims 11-14, 16, 51, and 52 are rejected under 35 U.S.C. 112, second paragraph, as failing to set forth the subject matter which applicant(s) regard as their invention.

a) Claims 11-14, 16, 51, and 52 are rejected under 35 U.S.C. 112, second paragraph, as being incomplete for omitting essential steps, such omission amounting to a gap between the steps. See MPEP § 2172.01. Claims 11-14, 16, 51 and 52 are indefinite for reciting incomplete method claims, which do not clearly set forth method steps and do not include a resolution step, which reads back on the preamble of the claimed method. Merely selecting a "host system" and expressing in said "host system" nucleic acids that encode an antibody does not result in a method of reducing the immunogenicity or toxicity. The claims should conclude with a step of inserting the IgG antibody into an organism and expressing the antibody in parallel with a glycosylated IgG antibody control, for example, thereby producing the method of reducing the immunogenicity or toxicity of an IgG antibody as required by the preamble, which recites "a method for reducing the immunogenicity or toxicity of an antibody or an antigen-binding antibody fragment of IgG class".

b) Claims 11-14, 16, 51, and 52 are indefinite for reciting "host system" in claims 11, 12, and 13. The phrase "host system" is not one which has a universally accepted meaning in the art nor is it one which has been adequately defined in the specification. The primary deficiency in the use of this phrase is the absence of an ascertainable meaning for said phrase. Since it is unclear how the "host system" is to aglycosylate the antibodies or antigen-binding antibody fragments referred to in the claims, there is no way for a person of skill in the art to ascribe a discrete and identifiable "host system" to said phrase. It is unclear what else is contemplated by "host system" and whether the "host system" for reducing the immunogenicity and toxicity of an antibody by removal of the carbohydrate groups consists of: only eukaryotic cells, only prokaryotic cells, eukaryotic hosts that are missing or have been genetically engineered to eliminate essential glycosylation enzymes, eukaryotic cells that have been treated with tunicamycin, cells that express the antibodies and the antibodies are modified post expression by oxidation or enzymatically, different cell types that are known to glycosylate mammalian proteins differently, or some combination of mutated antibody polynucleotides that remove N-linked glycosylation sites and host cells that preferentially N-link glycosylate, for examples. In the absence of a single defined art recognized meaning for the phrase "host system" and lacking a clear definition in the specification, one of ordinary skill in the art could not determine the metes and bounds of the claims.

c) Claims 11-14, 16, 51 and 52 are indefinite for reciting "reducing immunogenicity or toxicity" in independent claims 11 and 12. Immunogenicity is defined

on page 14 of the disclosure as "a measure of the ability of a targeting protein or therapeutic moiety to elicit an immune response when administered to a recipient". However, it is not clear how the reduced immunogenicity or toxicity is to be measured. Is the reduced immunogenicity or toxicity to be measured relative to the glycosylated antibody, or the glycosylated humanized antibody, or the partially glycosylated antibody, or to the mutated antibody, or to differentially glycosylated antibodies due to expression in different host systems. Furthermore, it is not clear whether reduced immunogenicity or toxicity means a partial reduction or complete abolishment of immunogenicity or toxicity.

d) Claims 11-14, 16, 51 and 52 are indefinite for reciting "nucleotide sequence comprising nucleic acids encoding an IgG antibody" in independent claims 11 and 12. It is unclear whether or not the nucleotide sequence encoding the IgG antibody actually has a glycosylation site in it. Does the nucleotide sequence encoding the IgG antibody have a glycosylation site present in the nucleotide sequence?

## *Claim Rejections - 35 USC § 102*

8. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (e) the invention was described in (1) an application for patent, published under section 122(b), by another filed in the United States before the invention by the applicant for patent or (2) a patent granted on an application for patent by another filed in the United States before the invention by the applicant for patent, except that an international application filed under the treaty defined in section 351(a) shall have the effects for purposes of this subsection of an application filed in the United States only if the international application designated the United States and was published under Article 21(2) of such treaty in the English language.
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

9. Claims 11-13 are rejected under 35 U.S.C. 102(e) as being anticipated by Whitlow et al (U.S. Patent 5,869,620, filed 2/1995, which has priority to at least 11/1992) as evidenced by Plückthun et al (Immunotechnology. 3:83-105, 1997).

Claims 11-13 recite a method of reducing the immunogenicity or toxicity of an antibody or antigen-binding antibody fragment of IgG class by selecting a "host system" that does not N-link or O-link glycosylate the antibody and expressing in said "host system" the nucleic acids encoding the IgG antibody or antigen-binding antibody fragment. Due to the indefinite nature of the claims (see 112 2nd above), the claims are interpreted as only having two active method steps in claims 11 and 12, drawn to selecting a "host system" that does not N-link glycosylate an antibody or antigen-binding antibody fragment and expressing in said "host system" nucleic acids encoding an antibody or antigen-binding antibody fragment.

Whitlow teaches a method of expressing antigen-binding proteins in *E. coli* and the antigen-binding proteins would be less immunogenic because of the lack of the Fc region (glycosylated or aglycosylated) and may be less immunogenic due to their smaller size (Whitlow et al, column 22, lines 29-62 and Figs. 1-6 and column 5, lines 3-6). Since the art meets the limitations of the active method steps in the claims, the art inherently anticipates the claimed method. As evidenced by Plückthun et al, bacteria are known in the art not to glycosylate proteins (see page 88 left column, lines 1-4).

10. Claims 11-13 are rejected under 35 U.S.C. 102(b) as being anticipated by Better et al (Science 240:1041-1043, 1988) as evidenced by Plückthun et al (Immunotechnology. 3:83-105, 1997).

The claims and their interpretation have been described supra.

Better et al teach a method of expressing and secreting a chimeric mouse-human Fab protein in *E. coli*, which lacks the biological functions mediated by glycosylation in the Fc region (unable to activate C and bind Fc receptors, making the antibody less immunogenic) and bacteria are known in the art not to glycosylate proteins as evidenced by Plückthun et al (see page 88 left hand column, lines 1-4). Since the art meets the limitations of the active method steps in the claims, the art inherently anticipates the claimed method.

11. Claims 11-13, 16, and 51 are rejected under 35 U.S.C 102(b) as being anticipated by Tao et al (J. of Immunology 143(8):2595-2601, 1989).

Claims 11-13 have been described supra.

Claims 16 and 51 further limit the method of reducing immunogenicity or toxicity of an antibody or an antigen-binding antibody fragment antibody of IgG class recited in claims 11-13 by mutating the nucleic acids encoding the IgG antibody or IgG antigen-binding antibody fragment to prevent N-linked glycosylation.

Tao et al teach a method of producing carbohydrate-depleted rabbit and murine Ig (see page 2595 right column, lines 7-9).

Tao et al teach removing the N-linked glycosylation site at Asn-297 by site-directed mutagenesis in the constant region of a chimeric mouse-human IgG (see page 2595 right column, lines 12-21, 40-50 and page 2596 left column, lines 1-8).

Tao et al teach that removal of the N-linked glycosylation site at Asn-297 renders IgG unable to activate C and bind to FcγRI on human monocytes and macrophages. Furthermore, Tao et al teach that aglycosylated IgG1 completely lost the ability to bind C1q, however with igG3 mutants there is only decreased C1q binding (see page 2595 right column, lines 24-31 and page 2598 left column, lines 2-19). Therefore, the mutated IgG molecules would be less immunogenic or toxic, since the aglycosylated IgG molecules did not activate C, did not bind to FcγRI on human monocytes and macrophages and had at least a reduced binding avidity towards C1q.

***Claim Rejections - 35 USC § 103***

12. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

The factual inquiries set forth in *Graham* **v.** *John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.

2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art.
4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

13. Claims 11, 12, and 16 are rejected under 35 U.S.C. 103(a) as being unpatentable over Nose et al (Proc. Natl. Acad. Sci. USA 80:6632-36, 1983) and further in view of Morrison et al (Proc. Natl. Acad. Sci. USA 81:6851-6855, 1984) and Duncan et al (Nature. 332(21):738-740, 1988).

The claims recite a method of reducing the immunogenicity or toxicity of an antibody or an antigen-binding fragment of IgG class by mutating the nucleic acids to prevent N-linked glycosylation and expressing the nucleic acids in a "host system" that does not N-link glycosylate the antibody or an antigen-binding antibody fragment. Due to the indefinite nature of the claims (see 112 2$^{nd}$ above), the claims are interpreted as only having two active method steps in claims 11 and 12, drawn to selecting a "host system" that does not N-link glycosylate an antibody or antigen-binding antibody fragment and expressing in said "host system" nucleic acids encoding an antibody or

antigen-binding antibody fragment, wherein the nucleic acids have been mutated to prevent N-linked glycosylation.

Nose et al teach the biological significance of carbohydrate chains in the constant region of a monoclonal antibody. Nose et al teach a method which produces a carbohydrate deficient monoclonal antibody (see abstract and page 6632 left column, lines 8-12 and right column, lines 25-32). Nose et al also teach that removal of the carbohydrate chains from antibody molecules had an impact on biological functions such as ADCC, binding to Fc receptors on macrophages (effector functions), complement activation and rapid elimination of antigen-antibody complexes (see page 6632 left column, lines 13-22 and page 6634 right column, lines 13-20, 30-35 and page 6635 left column, lines 1-3, 11-19, right column, lines 1-3, 15-20) and "the present studies and those involving actual modifications of the peptide parts of Ig molecules suggest the possibility of obtaining antibodies devoid of select biological functions" (see page 6636 lines 13-16). Nose does not teach deletion of the carbohydrate recognition site by site-directed mutagenesis. This deficiency is made up for in the teachings of Morrison et al and Duncan et al.

Morrison et al teach methods of producing monoclonal antibodies which are devoid of asparagine-linked glycosylation and the antibodies can be monoclonal or human (see page 6854, discussion) and human antibodies or near human antibodies (i.e. humanized) might decrease or eliminate the immunogenicity of antibodies used in vivo relative to mouse antibodies (see page 6854, bridging paragraph for left and right columns). Morrison et al also teach DNA methods to produce the antibodies and "It is

now feasible to shuffle exons or other kinds of directed mutagenesis to explore the human antibody molecules constant region structures required for carrying out diverse antibody functions" (see page 6854, right column lines 23-26) and Morrison et al teach the loss of the asparagine-linked carbohydrate moiety in the CH2 domain of IgG effects the biological effector functions such as complement-fixation are lost (see page 6853 left column, line 29, right column, lines 1-7).

Duncan et al teach a method of removing the N-linked glycosylation site at Asn-297 in the CH2 domain of the constant region of IgG by site-directed mutagenesis (see page 738 right column lines 5-16 and Fig. 1). Duncan et al teach that the Asn 297→ Ala mutation eliminates the ability of IgG to fix complement and reduced the antibody affinity for C1q (the first step in the complement cascade) about threefold (see page 738 right column, lines 21-27).

It would have been prima facie obvious to one of ordinary skill in the art at the time the claimed invention was made to have produced a method to eliminate the N-linked glycosylation site in the constant region of the humanized antibody by site-directed mutagenesis in order to modify the biological functions (i.e. reduced immunogenicity or toxicity) of the humanized antibody in view of Nose et al and Morrison et al and Duncan et al.

One of ordinary skill in the art would have been motivated to and had a reasonable expectation of success to have produced a method to eliminate the N-linked glycosylation site in the constant region of the antibody by site-directed mutagenesis to modify the biological functions of the antibody in view of Nose et al and Morrison et al

and Duncan et al because Nose et al teach a method which produces a carbohydrate deficient monoclonal antibody and removal of the carbohydrate chains from IgG molecules had an impact on biological function such as ADCC, binding to Fc receptors, complement activation and rapid elimination of antigen-antibody complex and "the present studies and those involving actual modifications of the peptide parts of IgG molecules suggest the possibility of obtaining antibodies devoid of select biological functions" (see page 6636 lines 13-16). In addition, one of ordinary skill in the art would have been motivated to and had a reasonable expectation of success to have produced a method to eliminate the N-linked glycosylation site in the constant region of the humanized antibody by site-directed mutagenesis to modify the biological functions of the humanized antibody in view of Nose et al and Morrison et al and Duncan et al because Morrison et al and Duncan et al teach that loss of the carbohydrate in the CH2 region (see Duncan et al, specifically, Asn 297→ Ala) results in loss of biological function of complement fixation and Morrison et al and Duncan et al teach recombinant DNA methods which produce antibodies that bind antigen and this (meaning the use of recombinant DNA techniques to produce antibodies in mammalian lymphoid cells) obviates the need for any post synthetic in vitro modification of the immunoglobulin polypeptide (see Morrison et al page 6854, right column). Therefore, it would have been obvious to use site-directed mutagenesis to produce an antibody that when purified would produce an antibody that was 100% aglycosylated rather than the 80-87% by the method of Nose et al (see table 1). Thus, it would have been obvious to one skilled in the art to produce a method that deletes the carbohydrate site in the

constant region of the antibody by using site-directed mutagenesis to modify the biological functions of the antibody in view of the teachings of Nose et al and Morrison et al and Duncan et al.

14. Claims 11-14, 16, 51 and 52 are rejected under 35 U.S.C. 103(a) as being unpatentable over Tao et al (J. of Immunology 143(8):2595-2601, 1989) as applied to claims 11-13, 16, and 51 above and further in view of Caron et al (Cancer Research 52:6761-67, 1992).

Claims 11-13, 16 and 51 have been described supra.

Claims 14 and 52 further limit the method of claims 11-13, 16 and 51 by reciting that the IgG antibody or IgG antigen-binding antibody fragment is a humanized antibody with its nucleic acid sequence mutated to prevent N-linked glycosylation.

Tao et al teach the biological significance of carbohydrate chains in the constant region of human IgG molecules. Tao et al teach a method of removing carbohydrate attachment at Asn-297 by site-directed mutagenesis in the CH-2 domain of the constant region of a chimeric mouse-human IgG (see page 2595 right column, lines 12-21, 40-50 and page2596 left column, lines 1-8). Tao et al teach that removal of the carbohydrate at Asn-297 in the CH-2 domain of the constant region renders IgG unable to activate C and bind to FcγRI on human monocytes and macrophages. Tao et al also teach that IgG1 and IgG3 were completely deficient in their ability to activate C and to bind human FCγRI; with aglycosylated IgG1 the ability to bind C1q is completely lost, however with IgG3 mutants there is only decreased C1q binding (see page 2595 right column, lines 24-31 and page 2598 left column, lines 2-19). Tao et al does not teach the deletion of

carbohydrate sites in humanized antibodies. This deficiency is made up for in the teachings of Caron et al.

Caron et al teach methods of producing humanized and chimeric IgG antibodies (see page 6761 right column, lines 4-16 and 43-54) and the humanized antibodies have the advantage of neutralizing HAMA responses (see page 6761 right column, lines 8-12). Caron et al teach that a loss of a carbohydrate binding site in the humanized variable region relative to the mouse variable region increased the avidity of binding (see page 6761 right column, lines 4-16, 43-54 and page 6765 right column, lines 49-56). Caron et al teach that the increased avidity of binding of the humanized antibodies enables the use of significantly smaller doses in vivo to achieve saturation of target (see page 6761 left column, lines 23-26 and page 6766 right column, lines 29-31). Caron et al teach that the humanized antibody HuG1 yielded 50% cell lysis and that HuG3 was consistently less efficient at CMC, achieving less than 50% maximum cell killing (see page 6764 left column, lines 1-4). Caron et al also teach that the humanized antibodies HuG1 and HuG3 showed less ADCC activity compared to the chimeric antibodies and suggest the difference is attributed to a change in a carbohydrate moiety attachment site on the IgG framework region that occurred during reconstruction (see page 6764 right column, lines 30-37 and page 6766 left column, lines 10-16).

It would have been prima facie obvious to one of ordinary skill in the art at the time the claimed invention was made to have produced a method to eliminate glycosylation sites in the constant and variable regions of a humanized IgG by site-

directed mutagenesis to avoid neutralizing HAMA responses, reduce immunogenicity, increase binding avidity and reduce the biological functions.

One of ordinary skill in the art would have been motivated to and had a reasonable expectation of success to have produced a method to eliminate glycosylation sites in the constant and variable regions of a humanized IgG by site-directed mutagenesis to avoid neutralizing HAMA responses, reduce immunogenicity, increase binding avidity and reduce the biological functions in view of Tao et al and Caron et al because Tao et al teach a method which produces a carbohydrate deficient IgG constant region and removal of the carbohydrate in the constant region had an impact on biological function such as C activation, binding to FcγRI on human monocytes and macrophages, and binding to C1q. In addition, one of ordinary skill in the art would have been motivated to and had a reasonable expectation of success to have produced a method to delete the carbohydrate recognition sites in the constant and variable regions of a humanized IgG to reduce biological function and increase avidity of binding in view of Tao et al and Canon et al because Tao et al teach that loss of the carbohydrate in the constant region (Asparagine-linked carbohydrate moiety in the CH2 domain; Asn 297) reduces biological function and Canon et al teach that carbohydrate removal in the variable region increased the binding avidity of a humanized IgG (see Canon et al page 6854, abstract and page 6766 right column, lines 26-31). Therefore, it would have been obvious to use site-directed mutagenesis to eliminate glycosylation sites in the constant and variable regions to produce a humanized IgG that neutralized HAMA responses, increased binding avidity so that the

humanized IgG could be used in smaller doses to achieve saturation, and reduce biological functions (i.e. C activation and binding to Fc receptors) of the humanized IgG, thereby reducing the overall immunogenicity and toxicity. Thus, it would have been obvious to one skilled in the art to produce a method that eliminates the glycosylation sites in the constant and variable regions of a humanized IgG by site-directed mutagenesis to avoid neutralizing HAMA responses, increase binding avidity and modify the biological functions of the humanized antibody, thereby reducing the immunogenicity and toxicity of the antibody in view of the teachings of Tao et al and Caron et al.

15. Claims 11-14, 16, 51 and 52 are rejected under 35 U.S.C. 103(a) as being unpatentable over Ma et al (Trends in Biotechnology 13:522-27, 1995) and further in view of Duncan et al (Nature. 332(21):738-40, 1988) and Caron et al (Cancer Research 52:6761-67, 1992).

Claims 11, 12 and 16 have been described supra.

Ma et al teach that plants can be used as bioreactors for large-scale production of correctly folded and assembled complete antibody molecules that are functionally identical to their mammalian counterpart (see page 522, abstract and left column, lines 19-32). Ma et al teach that for human therapy, the presence of plant-specific glycans might increase the immunogenicity of an antibody expressed in plants and suggest removing the N-linked glycosylation site to reduce immunogenicity (see page 523 right column, lines 4-11). Alternatively, Ma et al teach that mutant plants lacking enzymes involved in the glycosylation pathway could be used to produce antibodies that are

aglycosylated (see page 523 right column, lines 4-13). Ma et al teach that plants possess the ability to assemble full-length heavy chains with light chains to form complete antibodies, an important advantage over other recombinant expression systems (see page 522 right column, lines 2-13). Ma et al also suggest the possibility of using plants to exploit the production of full-length antibodies for engineering antibody molecules with altered Fc-mediated properties (see page 524 right column, lines 7-35). Ma does not teach elimination of the N-linked glycosylation site in the CH-2 domain of the constant region of a humanized IgG by site-directed mutagenesis. This deficiency is made up for in the teachings of Duncan et al and Caron et al.

Duncan et al and Caron et al have been described supra.

It would have been prima facie obvious to one of ordinary skill in the art at the time the claimed invention was made to have expressed complete humanized IgG antibodies in plants and eliminate the N-linked glycosylation site at Asn-297 in the CH-2 domain of the constant region of the humnized IgG by site-directed mutagenesis to avoid neutralizing HAMA responses, reduce the immunogenicity for human therapy, increase binding avidity, and modify the Fc-mediated biological functions of the antibody to further reduce the immunogenicity and toxicity in view of Ma et al and Duncan et al and Caron et al.

One of ordinary skill in the art would have been motivated to and had a reasonable expectation of success to have expressed complete humanized IgG antibodies in plants with the N-linked glycosylation site at Asn-297 in the CH-2 domain of the constant region of the humanized IgG eliminated by site-directed mutagenesis to

avoid neutralizing HAMA responses, reduce the immunogenicity for human therapy, increase binding avidity, and modify the Fc-mediated biological functions of the antibody to further reduce the immunogenicity and toxicity in view of Ma et al and Duncan et al and Caron et al because Ma et al teach that antibody expression in plants produces a plentiful supply of complete, functional antibody and for human therapy suggests removing the N-linked glycosylation site to reduce immunogenicity (see page 523 right column, lines 4-11) and plants can be exploited to produce antibody molecules with altered Fc-mediated biological functions (see page 524 right column, lines 6-9). In addition, one of ordinary skill in the art would have been motivated to have expressed humanized IgG antibodies in plants with the N-linked glycosylation site at Asn-297 in the CH-2 domain of the constant region of IgG eliminated by site-directed mutagenesis avoid neutralizing HAMA responses, reduce the immunogenicity for human therapy, increase binding avidity, and modify the Fc-mediated biological functions of the antibody to further reduce the immunogenicity and toxicity in view of Ma et al and Duncan et al and Caron et al because Duncan et al teach that loss of the carbohydrate at Asn-297 in the CH2 domain of the constant region of an IgG (see page 738 right column, lines 5-16 and Fig. 1) results in loss of complement fixation and reduced affinity for C1q and Canon et al teach that carbohydrate removal in the variable region increased the binding avidity of a humanized IgG. Therefore, it would have been obvious to use site-directed mutagenesis to produce a complete, functional humanized IgG with reduced immunogenicity and modified biological functions. Thus, it would have been obvious to one skilled in the art to have expressed complete, functional humanized IgG in plants

with the N-linked glycosylation site at Asn-297 in the CH-2 domain of the constant region eliminated by site-directed mutagenesis avoid neutralizing HAMA responses, to reduce immunogenicity for human therapy, increase binding avidity and modify the biological functions to further reduce the immunogenicity and toxicity of a humanized IgG in view of the teachings of Ma et al and Duncan et al and Caron et al.

Therefore, the invention as a whole was prima facie obvious to one of ordinary skill in the art at the time the invention was made, as evidenced by the references.

***Conclusion***

16. No claim is allowed.

17. Any inquiry concerning this communication or earlier communications from the examiner should be directed to David Blanchard, whose telephone number is (703) 605-1200. The examiner can normally be reached on Monday through Friday from 8:00 am to 4:30 pm. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Anthony Caputa, can be reached on (703) 308-3995. Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the Group receptionist whose telephone number is (703) 308-0196.

Papers related to this application may be submitted to Group 1600 by facsimile transmission. Papers should be faxed to Group 1600 via the PTO Fax Center located in Crystal Mall 1. The faxing of such papers must conform with the notice published in the

Official Gazette, 1096 OG 30 (November 15, 1989). The CM1 Fax Center telephone number is (703) 308-4242.

Respectfully,
David Blanchard.
703-605-1200

LARRY R. HELMS, PH.D
PRIMARY EXAMINER

Application No. 10/056,794

# NOTICE TO COMPLY WITH REQUIREMENTS FOR PATENT APPLICATIONS CONTAINING NUCLEOTIDE SEQUENCE AND/OR AMINO ACID SEQUENCE DISCLOSURES

The nucleotide and/or amino acid sequence disclosure contained in this application does not comply with the requirements for such a disclosure as set forth in 37 C.F.R. 1.821 - 1.825 for the following reason(s):

- [X] 1. This application clearly fails to comply with the requirements of 37 C.F.R. 1.821-1.825. Applicant's attention is directed to these regulations, published at 1114 OG 29, May 15, 1990 and at 55 FR 18230, May 1, 1990.
- [ ] 2. This application does not contain, as a separate part of the disclosure on paper copy, a "Sequence Listing" as required by 37 C.F.R. 1.821(c).
- [ ] 3. A copy of the "Sequence Listing" in computer readable form has not been submitted as required by 37 C.F.R. 1.821(e).
- [ ] 4. A copy of the "Sequence Listing" in computer readable form has been submitted. However, the content of the computer readable form does not comply with the requirements of 37 C.F.R. 1.822 and/or 1.823, as indicated on the attached copy of the marked -up "Raw Sequence Listing."
- [ ] 5. The computer readable form that has been filed with this application has been found to be damaged and/or unreadable as indicated on the attached CRF Diskette Problem Report. A Substitute computer readable form must be submitted as required by 37 C.F.R. 1.825(d).
- [ ] 6. The paper copy of the "Sequence Listing" is not the same as the computer readable from of the "Sequence Listing" as required by 37 C.F.R. 1.821(e).
- [ ] 7. Other: _____

## Applicant Must Provide:

- [X] An initial or substitute computer readable form (CRF) copy of the "Sequence Listing".
- [X] An initial or substitute paper copy of the "Sequence Listing", as well as an amendment directing its entry into the specification.
- [X] A statement that the content of the paper and computer readable copies are the same and, where applicable, include no new matter, as required by 37 C.F.R. 1.821(e) or 1.821(f) or 1.821(g) or 1.825(b) or 1.825(d).

For questions regarding compliance to these requirements, please contact:

For Rules Interpretation, call (703) 308-4216
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